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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT	:	Russell G. Higuchi	
SERIAL NO.	:	07/695,201	GROUP ART: to be assigned 187
FILED	:	May 2, 1991	EXAMINER: to be assigned
DOCKET NO.	:	2599	
TITLE	:	HOMOGENEOUS METHODS FOR NUCLEIC ACID AMPLIFICATION AND DETECTION	

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INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §1.56, §1.97, AND §1.98

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

The Examiner may find the following references and information, submitted in accordance with the duty of the disclosure under 37 C.F.R. §1.56, §1.97, and §1.98, material in the examination of the above-identified application. Copies of the references listed below and on the attached P.T.O. 1449 form are provided. The Examiner is requested to make these references of official record in the application.

The present invention provides a method for detecting a target nucleic acid in a sample. The novel methods for simultaneous nucleic acid amplification and detection enhance the speed and accuracy of prior detection methods and eliminate the need for sample processing following amplification. In a preferred embodiment, the method provides a modification of the polymerase chain reaction and utilizes DNA-binding agents whose fluorescence is enhanced upon binding double-stranded DNA.

Due to the number of publications cited on the accompanying P.T.O. 1449 form, the references are generally divided according to subject matter.

Alternative Fluorescence Based Methods for Detecting DNA

The following references relate to the use of fluorescent DNA binding agents for detecting DNA. According to these methods, the fluorescent dye is introduced to the target DNA after all in

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By Lisa Post

vitro nucleic acid polymerization reactions have been completed. None of these references teaches or suggests Applicant's claimed invention.

1. Sharp et al., 1973, Biochemistry 12:3055
2. Morrison et al., 1989, Anal. Biochem. 183:231-244
3. Glazer et al., 1990, Proc. Natl. Acad. Sci. USA 87:3851-3855
4. Mabuchi and Nishikawa, 1990, Nuc. Acids Res. 18(24):7461-7462
5. Oser and Valet, 1990, Angew. Chem. Int. Engl. 29(10):1167
6. European Patent Publication No. 070,685

Sharp et al. describe a method for detecting and sizing DNA fragments following gel electrophoresis. According to the method, ethidium bromide is included in the agarose gel matrix. Consequently, the gel staining and de-staining steps, required by previous methods, are avoided.

Morrison et al. describe a two probe method for detecting target DNA. One probe is labeled with fluorescein, the other probe, complementary to the first, is labeled with a quencher for fluorescein emission. The probes are allowed to anneal with denatured DNA containing the target sequence, and the amount of fluorescence is determined. Fluorescence increases to the extent that the fluorescein probe binds to unlabeled, complementary DNA rather than the complementary, quenching probe.

Glazer et al. describe a modified method for detecting electrophoresed DNA. According to the method, background flourescence is reduced by incubating the sample DNA with ethidium homodimer prior to electrophoresis. The dye is not included in the gel matrix and no subsequent staining procedure is required for visualization of the DNA fragments.

Mabuchi and Nishikawa describe a method for detecting DNA fragments based on the AT content of the nucleic acid segment. Two fluorochromes are used to stain size-fractionated DNA in an agarose gel. The selective binding properties of different fluorochromes for AT-rich regions are used to distinguish electrophoresed DNA fragments.

Oser and Valet describe a nucleic acid detection scheme that requires two oligonucleotide probes complementary to adjacent sites on a target. The probes are labeled differentially with either a salicylate or a DTPA ligand bearing a fluorescence emitter, Tb^{III}. Hybridization of both probes to the target provides steric proximity of the two labels resulting in a measurable increase in Tb^{III} fluorescence. The modified probes are prepared specifically for each target to be detected.

European Patent Publication No. 070,685 describes the use of fluorescent labeled probes in polynucleotide hybridization assays. According to the method, probes are prepared by attaching particular absorber-emitter moieties to the 3' and 5' ends of nucleic acid fragments. The fragments are capable of hybridizing to adjacent positions on a target DNA, so that, if both fragments are

hybridized, the proximity of the absorber and emitter moieties results in detectable emitter fluorescence.

Alternative DNA Binding Compounds

The following references characterize the interactions of a number of DNA-binding agents with double-stranded DNA. These references also describe methods for identifying compounds that intercalate double-stranded DNA. None of these references teaches or suggests Applicant's claimed invention.

7. Sobell and Jain, 1972, J. Mol. Biol. 68:21-34
8. Kapuseinski and Szer, 1979, Nuc. Acids Res. 6(112):3519-3535
9. Johnson and Hearst, 1981, Photochem. and Photobiol. 33:785-791
10. Khattar et al., 1990, JACS 112:4960
11. Searle and Embrey, 1990, Nuc. Acids Res. 18(13):3753-3762
12. U.S. Patent No. 4,119,521
13. U.S. Patent No. 4,257,774

Jain and Sobell describe that Actinomycin D forms a fluorescent complex with G-C rich DNA.

Kapuseinski and Szer describe the sequence specific interaction of DAPI with DNA. DAPI forms a fluorescent complex with AT rich sequences .

Johnson and Hearst describe the interaction between DNA and another photoreactive psoralen, 4-aminomethyle-4-5'8-trimethylpsoralen (AMT). Both the absorption at long wavelengths and fluorescence, decline upon intercalation of AMT into the DNA helix.

Khattar et al. describe a ruthenium complex that fluoresces when bound to double- stranded DNA. The complex displays no photoluminescence in aqueous solution at ambient temperatures but is intensely photoluminescent in the presence of DNA.

Searle and Embrey describe the sequence specific DNA interaction of a non-intercalating DNA binding agent. Hoechst 33258 exhibits altered fluorescence with increasing amount of DNA target. Hoechst 33258 preferentially binds A-T base pairs.

U.S. Patent No. 4,119,521 describes methods for preparing derivatives of activated polysaccharides useful as fluorescent DNA intercalating agents. The derivatives are included in an agarose gel matrix for electrophoresis and visualization of DNA fragments.

U.S. Patent No. 4,257,774 describes an assay for direct binding of fluorescent intercalators to DNA, e.g., ethidium salts, daunomycin, mepacrine and acridine orange, as well as 4'6-diamidino-α-phenylindole to quantitate the DNA. Fluorescence polarization is used to

characterize non-fluorescent DNA binding compounds which complete with the DNA binding dyes.

The Effects of DNA Binding Agents on Enzymatic Processes

The following references, all cited in the present patent application, characterize the effects of intercalating agents on nucleic acid polymerases.

14. Richardson, 1973, J. Mol. Biol. 78:703-714
15. Kornberg, 1974 in DNA Synthesis,W.H. Freman and Co., San Francisco, pp. 220-236
16. European Patent Publication No. 169,787

Richardson characterizes the inhibitory effects of ethidium bromide on various steps of the RNA synthesis reaction catalyzed by E. coli RNA polymerase. The specific inhibitory effects of other DNA binding agents are compared.

Kornberg describes a number of DNA binding agents, both intercalators and non-intercalators, and describes how each compound inhibits nucleic acid replication. At page 227, Kornberg specifically describes that ethidium bromide inhibits DNA replication. Chapter 7, section 14, pages 220-236 are included.

European Patent Publication No. 169,787 describes the use of DNA binding dyes as antibiotics due to of the inhibitory effects on nucleic acid replication processes that result from the agent binding to the template. The publication particularly describes the use of intercalating agents for blocking replication of influenza or herpes virus. Applicant notes that the publication is in French, and a translation is not provided. Should Examiner so request, Applicant will order an English translation for Examiner's review.

Related Patents and Applications

A number of commonly assigned patents and patent applications are cited in the present patent application and a concise explanation of each patent/application is provided below. Examiner will please note that several of the references listed are pending U.S. patent applications, and, as such, are not listed on the accompanying P.T.O. 1449 form. Copies of co-pending U.S. patent applications are not attached and Applicant believes that these documents are readily available to Examiner. However, should Examiner request copies, Applicant will promptly comply. Issued U.S. patents are listed on the accompanying P.T.O. Form 1449 and copies are provided.

U.S. Patent No./ Serial No.	Filing Date	Subject Matter
4,582,789	December 18, 1984	Psoralens for Labeling Nucleic Acids
4,683,195	February 7, 1986	PCR for Detection
4,683,202	October 25, 1985	PCR
4,889,818	June 17, 1987	Taq Polymerase
076,394	July 22, 1987	Labeled Primers for PCR
481,501	February 16, 1990	"Hot Start" Amplification Methods
563,758	August 6, 1990	Homogeneous Assay System
609,157	November 2, 1990	Sterilization Methods for PCR

U.S. Patent No. 4,582,789 describes several intercalating moieties including psoralens.

U.S. Patent Nos. 4,683,195 and 4,683,202 describe an amplification procedure, the polymerase chain reaction (PCR), for nucleic acids. The '195 patent specifically deals with detection of DNA sequences after amplification. The '202 patent describes the basic PCR process.

U.S. Patent No. 4,889,818 discloses the use of DNA polymerase of Thermus aquaticus (Taq polymerase) as the preferred DNA polymerase for PCR. The patent discloses methods for the purification and recombinant expression of Taq polymerase.

U.S. Serial No. 076,394 describes PCR-based methods for simultaneous amplification and labeling of a target nucleic acid. The methods require that at least one amplification primer is labeled.

U.S. Serial No. 481,501 describes methods for improving the specificity and convenience of PCR. The methods, referred to as "hot start" requires that at least one component that is essential for polymerization not be present until such time as the primer and template are both present, and the enzyme can bind to and extend the desired primer/template substrate.

U.S. Serial No. 563,758 describes a method for detecting amplified nucleic acids that employs the 5' to 3' nuclease activity of a nucleic acid polymerase to cleave annealed, labeled oligonucleotides from hybridized duplexes and release labeled oligonucleotide fragments for detection.

U.S. Serial No. 609,157 describes improved methods for reducing the effects of cross contamination of amplification reactions. The methods require the introduction of unconventional bases into the amplified product and exposing carryover to enzymatic and/or physical chemical treatment which effectively render the product incapable of serving as a template for subsequent amplifications.

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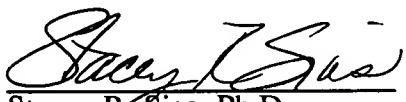
This Information Disclosure Statement under 37 C.F.R. §1.56, §1.97, and §1.98 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that this citation constitutes prior art under 35 U.S.C. §102 and §103.

Respectfully submitted,

By: 
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July 24, 1991

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